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Review

A review of therapeutic prospects of non-viral gene therapy in the retinal pigment epithelium

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ABSTRACT

Ocular gene therapy has been extensively explored in recent years as a therapeutic avenue to target diseases of the cornea, retina and retinal pigment epithelium (RPE). Adeno-associated virus (AAV)-mediated gene therapy has shown promise in several RPE clinical trials but AAVs have limited payload capacity and potential immunogenicity. Traditionally however, non-viral alternatives have been plagued by low transfection efficiency, short-term expression and low expression levels. Recently, these drawbacks have begun to be overcome by the use of specialty carriers such as polylysine, liposomes, or polyethyleneimines, and by inclusion of suitable DNA elements to enhance gene expression and longevity. Recent advancements in the field have yielded non-viral vectors that have favorable safety profiles, lack immunogenicity, exhibit long-term elevated gene expression, and show efficient transfection in the retina and RPE, making them poised to transition to clinical applications. Here we discuss the advancements in nanotechnology and vector engineering that have improved the prospects for clinical application of non-viral gene therapy in the RPE.

1. Introduction

The retinal pigment epithelium (RPE), a monolayer of cuboidal epithelial cells lining the back of the retina, performs multiple critical functions required for the maintenance of the neural retina and proper vision including nourishing and protecting the photoreceptor cells, absorbing excess light entering the retina, secreting growth factors, and recycling retinoids as part of the visual cycle [1]. In addition, tight junctions between RPE cells form the blood retina barrier and help the eye maintain its immune privilege [2]. Many blinding retinal disorders including Leber's congenital amaurosis (LCA) and retinitis pigmentosa (RP) occur due to mutations in RPE-specific genes, such as RPE65, bestrophin, and lecithin retinol acyltransferase (LRAT) (http://www.retina-international.org/sci-news/databases/mutation-database/). Choroidal neovascularization, age-related macular degeneration (AMD), and various other retinal diseases are also associated with defects in RPE-related structures, gene products, and metabolism [1]. Most often, in these cases blindness arises due to photoreceptor loss or malfunction secondary to RPE degeneration or functional defect.

Currently there are no curative therapies for RPE-associated diseases although many strategies are being explored. These include oral/intravitreal administration of retinoids, delivery of agents designed to alleviate ER stress, and cell transplantation. For example, treatment with 9-cis retinal, a precursor for the visual chromophore has mediated improvements in rod function in *rpe65*$^{-/-}$ mice [3] and in *lrat*$^{-/-}$ mice [4], and the pharmacological agent TUDCA (tauroursodexycholic acid) reduced ER stress and apoptosis resulting in significantly increased cone survival in *lrat*$^{-/-}$ mice [5]. Cell transplantation using human embryonic stem cells or retinal progenitor cells to replace dying photoreceptors and RPE is a rapidly expanding field, and recent results demonstrating successful formation of an optic-cup in 3D-embryonic stem cell culture [6,7] suggest that cell therapy has a highly promising future for ocular diseases. Due to limitations on cell integration and survival after transplantations much work remains to be done before this type of therapy finds clinical ocular application for transplantation of retinal neurons [8–10], however, progress in the RPE is more advanced and several initial clinical trials are underway or completed testing cell transplantation therapies for RPE-associated diseases [11] (Clinicaltrials.gov NCT00401713, NCT01691261, NCT01469832, NCT01344993).

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While these approaches have a bright future, particularly cell transplantation, by far the most well-developed field of research for the treatment of RPE-based diseases is gene therapy. Adeno-associated virus (AAV)-based gene delivery has been particularly fruitful. Many clinical trials testing AAV-based therapies for LCA associated with RPE65 and LRAT deficiency are underway based on extensive preliminary research using animal models [3,12,13]. In spite of these successes, there remain concerns about the safety of viral vectors, and production of neutralizing antibodies upon re-treatment [14], and although initial reports from the clinical trials were promising, concerns about damage from subretinal injection and limited rescue have prompted additional basic science research [15,16]. From a drug development standpoint, AAV vectors are limited by their small payload capacity (<5 kb of genetic cargo) [17] which cannot accommodate large genes or extensive promoter/regulatory elements such as the RPE gene *LRAT* [18] as well as photoreceptor genes such as *ABCA4* [19] (Stargardt's macular degeneration) and *USH2A* [20] (Usher syndrome type 2). Thus, development of alternative gene delivery strategies to complement AAVs for the treatment of RPE-associated diseases is a current research focus. Here we explore options for non-viral gene delivery to the RPE, discuss vector-based factors that contribute to the success of these strategies, and consider the prospects of these vectors for future clinical viability.

2. Non-viral gene therapy strategies

Successful non-viral gene delivery must overcome two traditional barriers; limited uptake into the cell and nucleus, and limited stability or transient gene expression once in the nucleus. Viral vectors have the advantage in cellular entry because they bind to the cellular receptors and co-receptors which help internalize and traffic them to the nucleus [21]. Such targeting and maintenance of plasmid vectors *in vivo* requires compaction with efficient gene carriers and modifications of the vector DNA. First we address options for gene carriers that have been tested in animal models of blindness including liposomes, organic polymers, nanoparticles, and plasmids.

2.1. Lipid cations (liposomes)

Liposomes are composed of lipid cations with hydrophobic head groups and hydrophilic tails and can incorporate negatively charged DNA or other drugs [22] (Fig. 1A). They can fuse with the plasma membrane for uptake making them exciting tools for gene and drug delivery [22–25]. Once inside the cell, the liposomes are processed via the endocytic pathway and the DNA is then released from the endosome/carrier into the cytoplasm (Fig. 1B) [26,27]. Liposome-mediated gene delivery is most successful in mitotic cells where DNA entry into the nucleus can take place during cell division. DNA has also been shown to enter the nucleus in the absence of mitosis but at a low frequency. In the past decade these lipid cations have been extensively studied and modified to reduce cytotoxicity and increase gene expression levels. Some of these liposomes are now being explored in clinical trials for periodontal disease and various types of cancer [28–31], and further research is focusing on improvements in shape, size, cytotoxicity, transfection efficiency, and biocompatibility, which may yield an efficacious method of gene delivery in the future.

Topical and intravitreal delivery of liposomes to the eye has been tried [32,33], however these delivery methods have yielded very low transfection efficiency for the retina and RPE. The inner limiting membrane and blood retina barrier severely limit passage of most gene delivery constructs including AAV after

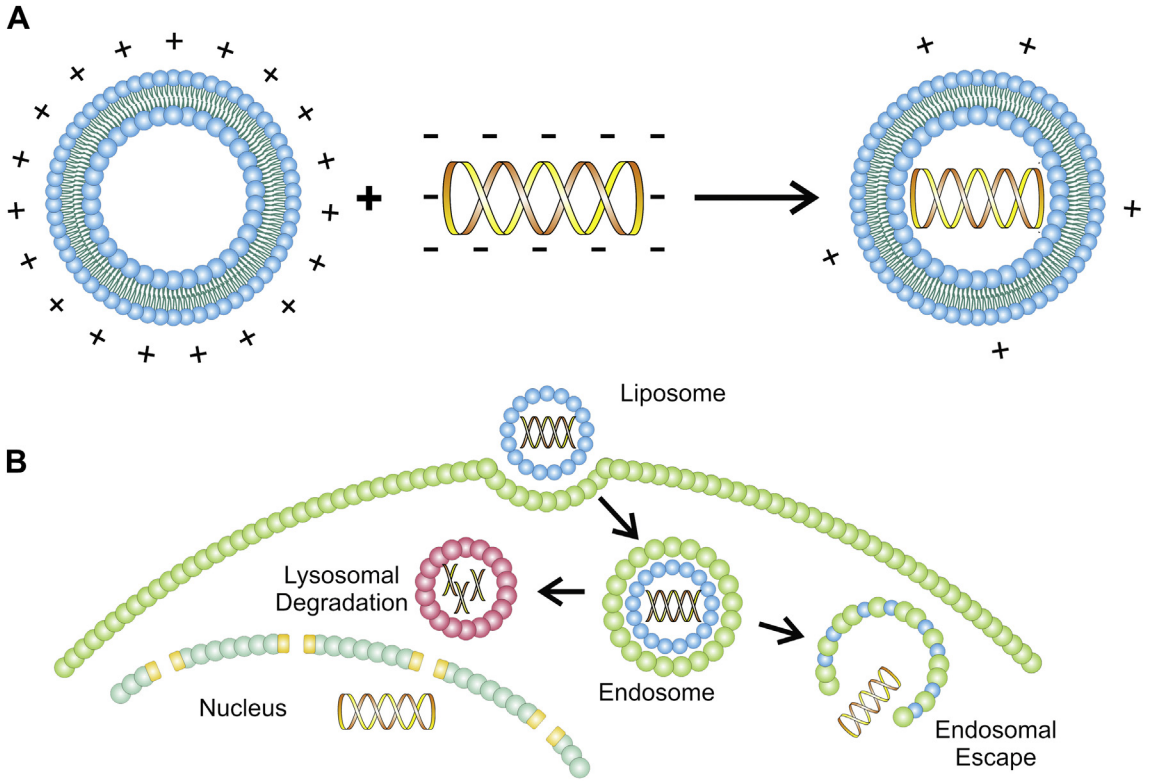

Fig. 1. Liposome-mediated transfection and endocytosis. A. Cationic lipids can form micelles that fuse with DNA molecules to form liposomes. B. These liposomes can be internalized by endocytosis, after which the DNA can be released into the cytosol or degraded in lysosomes.